# Appendix B: Excerpted cadmium analysis for dwarf wedgemussel and its host fish. EPA ESA Vermont WQS consultation for cadmium. (EPA 2020)

#### Cadmium Effects Assessment

- 1.1 Dwarf Wedgemussel (*Alasmidonta heterodon*)
- 1.1.1 Dwarf Wedgemussel Acute Cadmium Residential Exposure Effects Assessment

#### 1.1.1.1 Identifying Dwarf Wedgemussel Acute Cadmium Data

High quality species-level or genus-level acute cadmium toxicity data are not available for the dwarf wedgemussel. Family-level acute toxicity data were, therefore, used to determine an acute toxicity value (i.e.,  $LC_{50}$ ) of 65.58 µg/L (normalized to a total hardness of 100 mg/L as  $CaCO_3$ ) representative of the dwarf wedgemussel (Table 1). Acute toxicity data were obtained from Appendix A of USEPA (2016) and were used to derive the acute freshwater criterion. The Family Mean Acute Value (FMAV) is based on six Genus Mean Acute Values (GMAV). Five of the six GMAVs are composed of single Species Mean Acute Values (SMAVs), with the exception of the genus *Lampsilis*, which is based on four SMAVs.

Table 1. Data used to calculate the Unionidae FMAV representative of dwarf wedgemussel acute sensitivity to cadmium.

Common	Species	SMAV <sup>ab</sup> (µg/L)	Genus	GMAV <sup>ac</sup> (µg/L)	Family	FMAV <sup>ac</sup> (μg/L)
Neosho mucket	Lampsilis rafinesqueana	44.67				
Fatmucket	Lampsilis siliquoidea	35.73	Lampsilis	51.34		
Southern Fatmucket	Lampsilis straminea claibornensis	93.17	Lampsilis	31.34		
Yellow sandshell	Lampsilis teres	46.71				
Mussel	Actinonaias pectorosa	67.9	Actinonaias	67.9	Unionidae	65.58
Green floater	Lasmigona subviridis	68.51	Lasmigona	68.51		
Paper pondshell	Utterbackia imbecillis	71.76	Utterbackia	71.76		
Southern Rainbow	Villosa vibex	70.76	Villosa	70.76		
Dwarf wedge	Alasmidonta heterodon	N/A	Alasmidonta	N/A		

<sup>&</sup>lt;sup>a</sup> All acute toxicity data have been normalized to a hardness of 100 mg/L (as CaCO<sub>3</sub>) and expressed as total cadmium, consistent with Appendix A of the 2016 Cadmium 304(a) Aquatic Life Criteria document (USEPA 2016).

N/A: not available

<sup>&</sup>lt;sup>b</sup> SMAVs were obtained from Appendix A of the 2016 Cadmium 304(a) Aquatic Life Criteria document and organized based on taxonomy.

<sup>c</sup> GMAVs and FMAVs were calculated as the geometric mean of lower taxonomic-level geometric mean values, since these mean values are meant to represent the sensitivity for a particular taxon.

#### 1.1.1.2 Deriving LC<sub>50</sub> to LC<sub>5</sub> Acute Adjustment Factor

Raw acute toxicity data are only available from two tests out of the several that were used to calculate the surrogate FMAV representative of the dwarf wedgemussel [tests with 5-d old juvenile Neosho mucket (*Lampsilis rafinesqueana*) and fatmucket (*L. siliquoidea*) reported by Wang et al. (2010)]. The C-R models for the two tests, however, are unacceptable (see Cd-Acute-15 and Cd-Acute-16 in Appendix VT.2). The C-R model for the Neosho mucket (Cd-Acute-15) does not provide a unique solution and was flagged in TRAP for inadequate partials, while the C-R model for the fatmucket (Cd-Acute-16) is a poor fit. No other acute toxicity tests with C-R data are available for the Order Unionoida. As a result, EPA obtained and analyzed raw C-R data for all tests used to derive the acute cadmium criterion (underlined values in Appendix A of USEPA 2016; Table 2-2) where such data were reported or could be obtained to derive an acute vertebrate TAF or acute MAF, if necessary (i.e., if the vertebrate and invertebrate-level acute TAFs differ from one another).

Raw acute toxicity data were fit to C-R models using EPA's TRAP software to calculate LC<sub>50</sub> and corresponding LC<sub>5</sub> values for 69 tests representing 28 species (18 invertebrate and 10 vertebrates, including an amphibian). C-R models were excluded from TAF and MAF calculation if 1) models did not exhibit a unique solution and were flagged by TRAP as having inadequate partials; 2) models did not include observations in the region of interest which did not allow TRAP to accurately model a no-response plateau; and 3) models exhibited incongruities such as no or poor fit to key points or excessive noise in the C-R relationship. After exclusion of these unacceptable or questionable LC<sub>50</sub>:LC<sub>5</sub> ratios for use in calculating an acute MAF, 35 ratios remained resulting in seven genus-level LC<sub>50</sub>:LC<sub>5</sub> ratios for invertebrate species (arithmetic mean = 2.857  $\mu$ g/L, variance = 2.186  $\mu$ g/L) and six genus-level LC<sub>50</sub>:LC<sub>5</sub> ratios for vertebrate species (arithmetic mean = 2.106  $\mu$ g/L, variance = 0.2589  $\mu$ g/L). Analysis of the two arithmetic means via a two sample t-test assuming unequal variances ( $\alpha$  = 0.05) indicated the means are the same (t stat [1.259] < t critical for two tail [2.306]). As a result, the acute MAF was used to transform the *Acipenser* GMAV representative of dwarf wedgemussel to an acute low effect threshold concentration.

Table 2 provides the 13 genus-level LC<sub>50</sub>:LC<sub>5</sub> ratios used to derive the cadmium acute MAF. The acute MAF calculated as the geometric mean of all genus-level LC<sub>50</sub>:LC<sub>5</sub> ratios is 2.310 (see Appendix VT.1 [attached as a separate file: *Appendix\_VT\_Cadmium\_C\_R\_Data*] for raw toxicity test data, TRAP models and outputs for the 35 acute cadmium toxicity tests used to derive the acute MAF; Appendix VT.2 includes the raw toxicity data, TRAP models and output for all unacceptable and questionable acute cadmium toxicity tests).

Table 2. Acute LC<sub>50</sub>:LC<sub>5</sub> ratios from analysis of 35 high-quality acute cadmium toxicity tests with freshwater aquatic organisms used to derive an acute mean adjustment factor (MAF) for the dwarf wedgemussel.

Order	Family	Species	LC <sub>50</sub> (µg/L)	LC <sub>05</sub> (μg/L)	LC <sub>50</sub> : LC <sub>05</sub>	C-R Curve Label	Reference	Species-level TAF (LC50:LC05)	Genus-level TAF (LC <sub>50</sub> :LC <sub>05</sub> )
Tubificida	Naididae	Tubificid worm, Tubifex tubifex	56,141	27,732	2.024	Cd-Acute-2	Rathore and Khangarot 2002		
Tubificida	Naididae	Tubificid worm, Tubifex tubifex	26,650	10,289	2.590	Cd-Acute-5	Rathore and Khangarot 2002	2.278	2.278
Tubificida	Naididae	Tubificid worm, Tubifex tubifex	423.3	299.5	1.414	Cd-Acute-6	Rathore and Khangarot 2003	2.278	2.278
Tubificida	Naididae	Tubificid worm, Tubifex tubifex	6,463	1,778	3.634	Cd-Acute-8	Rathore and Khangarot 2003		
Basommatophora	Lymnaeidae	Pond snail (juvenile, stage II, 9 wk), Lymnaea stagnalis	1,735	718.0	2.416	Cd-Acute-9	Coeurdassier et al. 2004		
Basommatophora	Lymnaeidae	Pond snail (adult, 20 wk), Lymnaea stagnalis	1,670	1,051	1.590	Cd-Acute-10	Coeurdassier et al. 2004	2.016	2.016
Basommatophora	Lymnaeidae	Pond snail (juvenile, 25 mm), Lymnaea stagnalis	350.8	164.3	2.135	Cd-Acute-12	Pais 2012		
Basommatophora	Physidae	Snail (adult, 3.3-15 mm), <i>Physa acuta</i>	1,619	1,375	1.177	Cd-Acute-14	Woodard 2005	1.177	1.177
Diplostraca	Daphniidae	Cladoceran (<24 hr), Ceriodaphnia dubia	30.54	13.76	2.220	Cd-Acute-17	Shaw et al. 2006	2.220	2.220
Diplostraca	Daphniidae	Cladoceran (<24 hr), Daphnia magna	170.8	13.67	12.49	Cd-Acute-19	Shaw et al. 2006	4.580	4.580
Diplostraca	Daphniidae	Cladoceran (<24 hr), Daphnia magna	517.6	308.3	1.679	Cd-Acute-22	Perez and Beiras 2010	4.380	4.380
Decapoda	Cambaridae	Crayfish (adult), Orconectes virilis	6,007	2,427	2.475	Cd-Acute-30	Mirenda 1986	2.475	2.475
Ephemeroptera	Heptageniidae	Mayfly (nymph), Rhithrogena hageni	10,924	2,080	5.251	Cd-Acute-35	Brinkman and Vieira 2007; Brinkman and Johnston 2008	5.251	5.251
Salmoniformes	Salmonidae	Rainbow trout (8.8 g), Oncorhynchus mykiss	3.055	1.759	1.737	Cd-Acute-47	Phipps and Holcombe 1985	2.067	2.067

Order	Family	Species	LC50 (µg/L)	LC <sub>05</sub>	LC50: LC05	C-R Curve Label	Reference	Species-level TAF (LC50:LC05)	Genus-level TAF (LC50:LC05)
Salmoniformes	Salmonidae	Rainbow trout (juvenile, 18.3 g), Oncorhynchus mykiss	1.682	0.5849	2.876	Cd-Acute-48	Stubblefield 1990		
Salmoniformes	Salmonidae	Rainbow trout (36 g), Oncorhynchus mykiss	2.679	1.683	1.591	Cd-Acute-49	Davies et al. 1993		
Salmoniformes	Salmonidae	Rainbow trout (36 g), Oncorhynchus mykiss	7.052	3.007	2.345	Cd-Acute-53	Davies et al. 1993		
Salmoniformes	Salmonidae	Rainbow trout (fry, 1.0 g), Oncorhynchus mykiss	2.773	1.726	1.606	Cd-Acute-55	Davies and Brinkman 1994b		
Salmoniformes	Salmonidae	Rainbow trout (fry, 1.0 g), Oncorhynchus mykiss	2.152	1.116	1.928	Cd-Acute-58	Davies and Brinkman 1994b		
Salmoniformes	Salmonidae	Rainbow trout (fry, 2.5 g), Oncorhynchus mykiss	10.14	5.298	1.914	Cd-Acute-60	Davies and Brinkman 1994b		
Salmoniformes	Salmonidae	Rainbow trout (263 mg), Oncorhynchus mykiss	0.6500	0.3493	1.861	Cd-Acute-61	Stratus Consulting 1999	1	
Salmoniformes	Salmonidae	Rainbow trout (659 mg), Oncorhynchus mykiss	0.4134	0.2108	1.961	Cd-Acute-62	Stratus Consulting 1999		
Salmoniformes	Salmonidae	Rainbow trout (1,150 mg), Oncorhynchus mykiss	0.4634	0.2174	2.132	Cd-Acute-63	Stratus Consulting 1999	-	
Salmoniformes	Salmonidae	Rainbow trout (1,130 mg), Oncorhynchus mykiss	0.3528	0.2237	1.577	Cd-Acute-64	Stratus Consulting 1999	1	
Salmoniformes	Salmonidae	Rainbow trout (299 mg), Oncorhynchus mykiss	1.210	0.3198	3.784	Cd-Acute-65	Stratus Consulting 1999		
Salmoniformes	Salmonidae	Rainbow trout (289 mg), Oncorhynchus mykiss	2.548	1.042	2.445	Cd-Acute-66	Stratus Consulting 1999		
Salmoniformes	Salmonidae	Brown trout (fingerling, 22.4 g), Salmo trutta	2.732	0.9770	2.797	Cd-Acute-76	Stubblefield 1990	2.797	2.797
Salmoniformes	Salmonidae	Bull trout (0.200 g), Salvelinus confluentus	0.9828	0.4530	2.169	Cd-Acute-79	Stratus Consulting 1999		
Salmoniformes	Salmonidae	Bull trout (0.221 g), Salvelinus confluentus	0.9994	0.3656	2.734	Cd-Acute-80	Stratus Consulting 1999	2.402	2.402
Salmoniformes	Salmonidae	Bull trout (0.0842 g), Salvelinus confluentus	3.200	1.254	2.552	Cd-Acute-82	Stratus Consulting 1999	2.402	2.402
Salmoniformes	Salmonidae	Bull trout (0.0727 g), Salvelinus confluentus	5.942	2.700	2.201	Cd-Acute-83	Stratus Consulting 1999		

			LC50	LC <sub>05</sub>	LC50:	C-R Curve		Species-level TAF	Genus-level TAF
Order	Family	Species	(µg/L)	(μg/L)	LC <sub>05</sub>	Label	Reference	(LC50:LC05)	$(LC_{50}:LC_{05})$
Cypriniformes	Cyprinidae	Red shiner (adult, 0.80-2.0 g), <i>Cyprinella lutrensis</i>	6,731	4,903	1.373	Cd-Acute-85	Carrier 1987; Carrier and Beitinger 1988a	1.373	1.373
Cypriniformes	Cyprinidae	Zebrafish (adult),  Danio rerio	15,631	8,012	1.951	Cd-Acute-86	Vergauwen 2012; Vergauwen et al. 2013	1.710	1.710
Cypriniformes	Cyprinidae	Zebrafish (adult),  Danio rerio	12,384	8,263	1.499	Cd-Acute-87	Vergauwen 2012; Vergauwen et al. 2013	1./10	1.710
Anura	Pipidae	African clawed frog, Xenopus laevis	3,314	1,447	2.290	Cd-Acute-101	Sunderman et al. 1991	2.290	2.290

#### 1.1.1.3 Calculating Dwarf Wedgemussel Acute Cadmium Low Effect Threshold

Dividing the estimated dwarf wedgemussel LC<sub>50</sub> value (65.58  $\mu$ g/L) by the acute MAF (2.310) results in an acute low effect threshold concentration of 28.39  $\mu$ g/L (normalized to a hardness of 100 mg/L as CaCO3).

#### 1.1.1.4 Dwarf Wedgemussel: Acute Cadmium Effects Determination

The acute cadmium CMC at a hardness of 100 mg/L as  $CaCO_3$  ( $1.9 \mu\text{g/L}$  total Cd), is nearly 15 times lower than the dwarf wedgemussel acute cadmium low effect threshold of  $28.39 \mu\text{g/L}$  total cadmium. The dwarf wedgemussel acute low effect threshold concentration, calculated as described above based on data from continuous laboratory exposures, is greater than the corresponding criterion magnitude. As a result, refined assessment and consideration of the criterion duration is not necessary, and approval of the acute freshwater cadmium water quality standard may affect, but is Not Likely to Adversely Affect (NLAA) the dwarf wedgemussel.

## 1.1.2 Dwarf Wedgemussel Chronic Cadmium Residential Exposure Effects Assessment 1.1.2.1 Identifying Chronic Data

High quality species-level or genus-level chronic toxicity data are not available for the dwarf wedgemussel. Family-level chronic toxicity data were, therefore, used to determine a chronic toxicity value (i.e.,  $EC_{20}$ ) of 11.29 µg/L (normalized to a total hardness of 100 mg/L as  $CaCO_3$ ) representative of the dwarf wedgemussel (Table 3). The Unionidate Family Mean Chronic Value (FMCV) is based on a single chronic toxicity assay with the fatmucket mussel (endpoint = juvenile survival). This assay is the only mussel data used to derive the chronic criterion. Unionidae chronic toxicity data were obtained from Appendix C of USEPA (2016) and were used to derive the chronic criterion.

Table 3. Data used to derive the FMCV representative of dwarf wedgemussel sensitivity to cadmium.

Common	Species	SMCV a b (µg/L)	Genus	GMCV <sup>a</sup> (µg/L)	Family	FMCV <sup>a</sup> (µg/L)
Fatmucket	Lampsilis siliquoidea	11.29	Lampsilis	11.29	I Indiani dan	11.29
Dwarf wedge	Alasmidonta heterodon	N/A	Alasmidonta	N/A	Unionidae	11.29

<sup>&</sup>lt;sup>a</sup> Chronic toxicity data have been normalized to a hardness of 100 mg/L as CaCO<sub>3</sub> and expressed as total cadmium, consistent with Appendix C of the 2016 Cadmium 304(a) Aquatic Life Criteria document (USEPA 2016).

#### 1.1.2.2 Deriving EC<sub>20</sub> to EC<sub>5</sub> Chronic Adjustment Factor

Raw chronic toxicity data are available from the same test (Wang et al. 2010) used to calculate the Unionidae FMCV representative of the dwarf wedgemussel; however, the underlying C-R model (Cd-Chronic-9) lacks partial effects and does not provide a unique solution resulting in questionable EC<sub>x</sub> estimates, particularly when estimating low-level effects concentrations (see Appendix VT.4). No other chronic toxicity tests with C-R data are available for the Order Unionoida.

<sup>&</sup>lt;sup>b</sup> The single Species Mean Chronic Value (SMCV) used to derive the FMCV was obtained from Appendix C of the 2016 Cadmium 304(a) Aquatic Life Criteria document.

As a result, EPA obtained and analyzed raw C-R data for all tests used to derive the chronic criterion (USEPA 2016 Appendix C underlined values) where such data were reported or could be obtained to derive a chronic vertebrate TAF or chronic MAF, if necessary (i.e., if the vertebrate and invertebrate-level chronic TAFs differ from one another).

Raw chronic toxicity data were fit to C-R models using EPA's TRAP software to calculate EC<sub>20</sub> and corresponding EC<sub>5</sub> values for 40 tests representing 17 species (8 invertebrate and 9 fish species). C-R models were excluded from TAF and MAF calculation if 1) models did not exhibit a unique solution and were flagged by TRAP as having inadequate partials; 2) models did not include observations in the region of interest which did not allow TRAP to accurately model a no-response plateau; and 3) models exhibited incongruities such as no or poor fit to key points or excessive noise in the C-R relationship. After exclusion of unacceptable or questionable EC<sub>20</sub>:EC<sub>5</sub> ratios, 13 ratios remained resulting in three genus-level EC<sub>20</sub>:EC<sub>5</sub> ratios for invertebrate species (arithmetic mean = 1.779  $\mu$ g/L, variance = 0.07706  $\mu$ g/L) and four genus-level EC<sub>20</sub>:EC<sub>5</sub> ratios for vertebrate species (arithmetic mean = 1.332  $\mu$ g/L, variance = 0.008872  $\mu$ g/L). Analysis of the two means via a two-sample t-test assuming unequal variances ( $\alpha$  = 0.05) indicated that the means are the same (t stat [2.677] < t critical for two tail [4.303]). As a result, the chronic MAF was used to transform the GMCV applicable dwarf wedgemussel to a chronic low effect threshold concentration.

Table 4 provides the seven genus-level EC<sub>20</sub>:EC<sub>5</sub> ratios used to derive the chronic MAF. Individual test ratios ranged from 1.229 to 2.097. The chronic MAF calculated as the geometric mean of all genus-level EC<sub>20</sub>:EC<sub>5</sub> ratios is 1.502 (see Appendix VT.3 [attached as a separate file: *Appendix\_VT\_Cadmium\_C\_R\_Data*] for raw toxicity test data, TRAP models and outputs for the 13 chronic cadmium toxicity tests used to derive the chronic MAF; Appendix VT.4 includes the raw toxicity data, TRAP models and outputs for all unacceptable and questionable cadmium toxicity tests).

Table 4. Chronic EC<sub>20</sub>:EC<sub>5</sub> ratios from analysis of 13 high-quality chronic cadmium toxicity tests with freshwater aquatic organisms used to derive a chronic cadmium MAF representative of the dwarf wedgemussel.

Order	Family	Species	EC <sub>20</sub> (μg/L)	EC <sub>05</sub> (μg/L)	EC <sub>20</sub> : EC <sub>05</sub>	C-R Curve Label	Reference	Species-level TAF (EC <sub>20</sub> :EC <sub>05</sub> )	Genus-level TAF (EC <sub>20</sub> :EC <sub>05</sub> )
N/Aª	Aeolosomatidae	Oligochaete, Aeolosoma headleyi	57.35	27.35	2.097	Cd-Chronic-1	Niederlehner et al. 1984	2.097	2.097
Diplostraca	Daphniidae	Cladoceran, Ceriodaphnia dubia	4.940	3.352	1.474	Cd-Chronic-12	Southwest Texas State University 2000	1 504	1.584
Diplostraca	Daphniidae	Cladoceran, Ceriodaphnia dubia	5.505	3.235	1.702	Cd-Chronic-13	Southwest Texas State University 2000	1.584	1.384
Diplostraca	Daphniidae	Cladoceran, Daphnia magna	0.2118	0.1059	2.000	Cd-Chronic-15	Chapman et al. Manuscript	1.657	1 657
Diplostraca	Daphniidae	Cladoceran, Daphnia magna	6.166	4.489	1.374	Cd-Chronic-17	Bodar et al. 1988b	1.03/	1.657
Salmoniformes	Salmonidae	Rio Grande cutthroat trout Oncorhynchus clarkii virginalis	2.354	1.659	1.419	Cd-Chronic-24	Brinkman 2012	1.419	
Salmoniformes	Salmonidae	Rainbow trout, Oncorhynchus mykiss	2.283	1.774	1.287	Cd-Chronic-26	Davies et al. 1993		1.365
Salmoniformes	Salmonidae	Rainbow trout, Oncorhynchus mykiss	4.956	3.719	1.333	Cd-Chronic-27	Davies et al. 1993	1.312	1.303
Salmoniformes	Salmonidae	Rainbow trout, Oncorhynchus mykiss	4.315	3.272	1.319	Cd-Chronic-28	Davies et al. 1993		
Salmoniformes	Salmonidae	Brown trout, Salmo trutta	5.187	4.221	1.229	Cd-Chronic-42	Brinkman and Hansen 2004a; 2007	1.229	1.229
Cyprinodontiformes	Cyprinodontidae	Flagfish, Jordanella floridae	5.018	3.470	1.446	Cd-Chronic-48	Spehar 1976	1.446	1.446
Scorpaeniformes	Cottidae	Mottled sculpin,  Cottus bairdii	1.762	1.329	1.326	Cd-Chronic-52	Besser et al. 2007	1 200	1 200
Scorpaeniformes	Cottidae	Mottled sculpin, Cottus bairdii	1.285	1.026	1.252	Cd-Chronic-53	Besser et al. 2007	1.289	1.289

<sup>&</sup>lt;sup>a</sup> N/A; not available, no order listed in the Integrated Taxonomic Information System (www.itis.gov) for the species.

1.1.2.3 Calculating Dwarf Wedgemussel Chronic Cadmium Low Effect Threshold Dividing the estimated dwarf wedgemussel EC<sub>20</sub> value (11.29  $\mu$ g/L; family-level surrogate) by the chronic MAF (1.502) results in a chronic low effect threshold concentration of 7.517  $\mu$ g/L (total cadmium, normalized to a hardness of 100 mg/L as CaCO<sub>3</sub>).

### 1.1.2.4 Dwarf Wedgemussel: Chronic Cadmium Effects Determination

The cadmium CCC of  $0.79~\mu g/L$  total cadmium (at a hardness of 100~mg/L as  $CaCO_3$ ) is over 9.5 times lower than the dwarf wedgemussel chronic cadmium low effect threshold concentration of  $7.517~\mu g/L$  total cadmium. The dwarf wedgemussel chronic low effect threshold concentration, based on continuous laboratory exposures, is greater than the corresponding criterion magnitude. As a result, refined assessment and consideration of the criterion duration is not necessary, and approval of the chronic cadmium freshwater quality standard may affect, but is Not Likely to Adversely Affect (NLAA) the dwarf wedgemussel.

#### 1.1.3 Dwarf Wedgemussel, Prey Effects Assessment

The dwarf wedgemussel filters phytoplankton and zooplankton from the water column as a primary food source, with phytoplankton being relatively insensitive to acute and chronic cadmium exposures. For example, USEPA (2016) states, "Available data for aquatic plants and algae were reviewed to determine if they were more sensitive to cadmium than aquatic animals... Effect concentrations for freshwater plants and algae were well above the freshwater criteria...and it was therefore unnecessary to develop criteria based on the toxicity of cadmium to aquatic plants..." Acute toxicity data used to derive the acute freshwater cadmium criterion (Table 7 of USEPA 2016) indicate fish are the most sensitive to acute cadmium exposures, with pelagic crustaceans (e.g., zooplankton) being less sensitive and, therefore, adequately protected from acute cadmium exposures. Pelagic crustaceans (Hyalella and Ceriodaphnia) did comprise the two most-sensitive genera to chronic cadmium exposures (Table 9 of USEPA 2016); however, a large portion of individuals within the most sensitive genera are not anticipated to be affected because chronic toxicity values (i.e., EC<sub>20</sub> values) are based on exposure durations significantly longer (e.g., 7 to 28 days for invertebrate tests) than the chronic criterion duration (i.e., 4 days). Further, chronic effects on a large portion of zooplankton (which is not an anticipated effect of the proposed actions) would translate minimally to the dwarf wedgemussel prey base because mussels also rely on phytoplankton, which is tolerant to cadmium exposures (USEPA 2016).

Because criteria are derived to protect the broad aquatic community (including zooplankton and phytoplankton) and the dwarf wedgemussel is not a specialized feeder, instead relying on a range of pelagic organisms, EPA approval of Vermont acute and chronic freshwater cadmium standards may affect, but is not likely to adversely affect (NLAA) the dwarf wedgemussel through effects to its prey.

#### 1.1.4 Dwarf Wedgemussel, Host Fish Effects Assessment

The dwarf wedgemussel relies on host fish such as mottled sculpin to support the glochidia stage of its development. Dwarf wedgemussel reproduction and survival may be affected if host fish populations, on which glochidia may rely during the parasitic portion of the mussel's life cycle, were limited as a result of cadmium exposures specified by the acute and chronic criteria magnitude and duration. The objective of this refined effects assessment was to determine if

dwarf wedgemussel host fish are sensitive to cadmium at exposure magnitudes and durations specified by USEPA (2016).

#### Methods:

The residential exposure effects assessment for the dwarf wedgemussel focused on identifying acute and chronic low effect thresholds (i.e., acute LC<sub>5</sub> and chronic EC<sub>5</sub>) that were species specific. Rather than a species-specific approach to identify possible effects to host fish, EPA compiled acute and chronic cadmium toxicity values for host fish species (or surrogate species <sup>1</sup>) to derive acute and chronic species sensitivity distributions (SSDs). Acute and chronic SSDs were then used to calculate acute and chronic hazard concentration at the 5<sup>th</sup> percentile (HC<sub>5</sub>) that represent a concentration that was protective of low-level effects to 95% of fish genera that may be dwarf wedgemussel host fish.

• Identification of Dwarf Wedgemussel Host Fish:

Dwarf wedgemussel host fish species were initially identified at the suggestion of USFWS to consider sculpin species as potential hosts (personal communication between USFWS New England Field Office and EPA Region 1, Sept. 2019). The suggestion by USFWS was further supplemented by searching the USFWS Environmental Conservation Online System (ECOS) for recent technical documents pertaining to the dwarf wedgemussel that contained host fish information. USFWS (2019; Table 3 of the USFWS 5-year Review; Table 5 below) provided a review of all host fish species. Species in Table 5 were considered as possible host fish species for the parasitic life stage of the mussel.

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<sup>&</sup>lt;sup>1</sup> Fish species from the 2016 acute and chronic cadmium criteria species sensitivity distribution were retained in the dwarf wedgemussel host fish-specific SSDs if they were in the same Order of a host fish. Species in the same Order respond similarly to pollutant exposures, especially for cadmium, which does not have a targeted mode of action and generally affects all freshwater fish species similarly by disrupting calcium homeostasis and causing oxidative damage on gills (USEPA 2016).

Table 5. Host fish species, and supporting data and documentation.<sup>a</sup>

Potential DWM Fish Hosts Evaluated	Lab or Field	Glochidia Origin	Reference
tessellated darter (Etheostoma olmstedi), johnny darter (E. nigrum), and mottled sculpin (Cottus bairdi)	Lab	Tar River, NC	Michaelson and Neves 1995
slimy sculpin (C. cognatus), Atlantic salmon (Salmo salar) juveniles and parr	Lab	Ashuelot River, NH	Wicklow 2004
tessellated darter	Field	Mill River, MA	McLain and Ross 2005
pirate perch (Aphredoderus sayanus), redbreast sunfish (Lepomis auritus), green sunfish (L. cyanellus), bluegill (L. macrochirus), bluehead chub (Nocomis leptocephalus), highfin shiner (Notropis altipinnis), swallowtail shiner (Notropis procne), white shiner (Luxilus albeolus), pinewoods shiner (Lythrurus matutinus), fantail darter (E. flabellare), johnny darter, chainback darter (Percina nevisense), Roanoke darter (P. roanoka)	Lab	Tar River, NC	Levine et al. 2011
tessellated darter	Field	Ashuelot River, NH, Flat Brook, NJ	St. John White et al. 2017
tessellated darter, slimy sculpin, and Atlantic salmon parr	Lab	Ashuelot River, NH	St. John White et al. 2017
tessellated darter, slimy sculpin, mottled sculpin, shield darter (Percina peltata), Atlantic salmon parr, shield darter (Percina peltata), banded killifish (Fundulus diaphanus)	Lab	Flat Brook, NJ	St. John White et al. 2017
(poor hosts), brown trout (Salmo trutta) (poor hosts), and striped bass (Morone saxatillis)	S		S
Atlantic salmon parr	Lab	Mill River, Neversink River	St. John White et al. 2017

<sup>a</sup>Table obtained directly from USFWS (2019) that outlines dwarf wedgemussel host fish species. Note, blank spaces in the second to last row were the result of compiling the table from USFWS (2019) which spanned two pages. The second to last row represents lab data from Flat Brook, NJ from St. John White et al. (2017).

• Cadmium Toxicity Data Acquisition & Derivation of Acute and Chronic HC<sub>5</sub> Values:

Host fish species in Table 5 were cross walked with the 2016 cadmium criteria acute and chronic SSDs (see Table 7 and Table 9 of USEPA [2016], respectively) to identify host species (or surrogate species within in the same Order as host species<sup>3</sup>) with high quality acute and/or chronic cadmium toxicity data (see Tables 6 and 8). All species that were not within the same Order as a host fish species were removed from the acute and chronic SSDs to derive host fish-specific SSDs. The host fish-specific SSDs were compiled using acute and chronic toxicity data that had been normalized to a hardness of 100 mg/L (USEPA 2016). The acute and chronic host fish-specific SSDs contained species that served as direct hosts but also contained species that were retained to provide surrogate acute and chronic values for untested direct host species.

The chronic host fish-specific SSD was used to calculate a chronic HC<sub>5</sub> (USEPA 1985), representing 20% chronic effects to the  $5^{th}$  centile of sensitive host fish genera under long-term exposure scenarios (USEPA 1985). The acute host fish-specific SSD was used to calculate a final acute value (FAV) at the fifth percentile of the distribution. The FAV, representing 50% acute effects to the  $5^{th}$  centile of sensitive genera, was then divided by 2.0 to calculate the acute HC<sub>5</sub>. Dividing the FAV by 2.0 results in an HC<sub>5</sub> that is representative of low-level effects (e.g., 0 – 10% effects; indistinguishable from unexposed control organisms) to the  $5^{th}$  centile of sensitive genera (USEPA 1985).

• Comparing Dwarf Wedgemussel Acute and Chronic Host Fish HC<sub>5</sub> to Criteria Magnitudes

The acute  $HC_5$  was compared to the acute cadmium criterion magnitude (at hardness = 100 mg/L). If the acute  $HC_5$  was greater than the acute criterion magnitude, then the host fish species were considered adequately protected from acute cadmium exposures by the acute cadmium criterion (USEPA 2016). Similarly, the chronic  $HC_5$  was compared to the chronic cadmium criterion magnitude (at hardness = 100 mg/L). If the chronic  $HC_5$  was greater than the chronic criterion magnitude, then host fish species were considered adequately protected from long-term continuous chronic cadmium exposures by the chronic cadmium criterion (USEPA 2016).

#### Results:

The acute dwarf wedgemussel host species-specific SSD contained 30 species across 22 genera (Table 6). The chronic host species-specific SSD contained 15 species across 10 genera (Table 8). Because both SSDs contain less than 59 genera, the acute and chronic HC<sub>5</sub> values are both based on the four most sensitive genera (USEPA 1985).

The acute dwarf wedgemussel host species-specific HC<sub>5</sub> (hardness = 100 mg/L) was  $2.1 \mu\text{g/L}$  total cadmium (Table 7), slightly greater than the acute criterion magnitude of  $1.9 \mu\text{g/L}$  total cadmium. The chronic host species-specific HC<sub>5</sub> (hardness = 100 mg/L) was  $1.2 \mu\text{g/L}$  total cadmium (Table 9), greater than the chronic criterion magnitude of  $0.79 \mu\text{g/L}$  total cadmium.

#### Discussion and Conclusions:

Both the acute and chronic dwarf wedgemussel host species-specific HC<sub>5</sub> values were greater than their corresponding criteria magnitudes, indicating host fish species are tolerant to acute and chronic cadmium exposure magnitudes and durations specified by the cadmium criteria (USEPA 2016). Consequently, dwarf wedgemussel glochidia will not experience biological effects through reductions in host fish if cadmium were to exist at criteria magnitudes in all Vermont freshwaters for extended periods of time.

Moreover, the water quality standard approval action does not imply that cadmium will exist at criteria magnitudes in all Vermont freshwaters for extended periods of time. Rather, it's a simplified and conservative assumption used in this assessment as a conservative screening-level approach. For example, criteria are derived from tests that expose organisms to continuous cadmium concentrations for durations that are significantly longer than those specified in the acute and chronic criteria. Consequently, the acute and chronic cadmium criteria magnitudes are based on conservative exposure assumptions. For example, acute effect concentrations are inherently linked to exposure duration; the longer organisms are exposed to a particular pollutant, the lower (e.g., appear more sensitive) the observed acute effect concentration is anticipated to be (up to an incipient lethal concentration). Therefore, the acute cadmium criterion magnitude and corresponding one-hour duration are conservative, considering the acute criterion magnitude is based on 96-hour continuous exposure toxicity tests. As such, results of this refined effects assessment, based on the conservative screening-level exposure assumptions, ensure effects to dwarf wedgemussel host fish species (and resultant effects to the mussel) are not likely to occur.

Consequently, EPA approval of Vermont acute and chronic freshwater cadmium standards may affect, but is not likely to adversely affect (NLAA) the dwarf wedgemussel through effects to its host fish.

Table 6. Ranked freshwater genus mean acute values (GMAV) for species within the same order as dwarf wedgemussel fish host species.<sup>a</sup>

Rank <sup>b</sup>	Genus Mean Acute Value (µg/L)	Order	Species	SMAV adjusted to 100 hardness (µg/L)	Reason retained
22	30,781	Cypriniformes	Common carp, Cyprinus carpio	30,781	In the same family as 5 fish hosts
21	26,837	Perciformes	Nile tilapia, Oreochromis niloticus	66,720	In the same order as 5 fish hosts
-	-	Perciformes	Mozambique tilapia, Oreochromis mossambica	10,795	In the same order as 5 fish hosts
20	12,100	Cyprinodontiformes	Mosquitofish, Gambusia affinis	12,100	In the same order as 1 fish host
19	7,752	Perciformes	Green sunfish, Lepomis cyanellus	6,276	Direct host
-	-	Perciformes	Bluegill, Lepomis macrochirus	9,574	Direct host
18	7,716	Cypriniformes	Red shiner, Cyprinella lutrensis	7,716	In the same family as 5 fish hosts
17	6,808	Perciformes	Yellow perch, Perca flavescens	6,808	In the same family as 6 fish hosts
16	5,947	Cypriniformes	White sucker, Catostomus commersonii	5,947	In the same order as 5 fish hosts
15	5,583	Cyprinodontiformes	Flagfish, Jordanella floridae	5,583	In the same order as 1 fish host
14	4,929	Cyprinodontiformes	Guppy, Poecilia reticulata	4,929	In the same order as 1 fish host
13	2,967	Cypriniformes	Zebrafish,  Danio rerio	2,967	In the same family as 5 fish hosts
12	1,656	Cypriniformes	Goldfish, Carassius auratus	1,656	In the same family as 5 fish hosts
11	1,582	Cypriniformes	Fathead minnow, Pimephales promelas	1,582	In the same family as 5 fish hosts
10	651.3	Salmoniformes	Lake whitefish, Coregonus clupeaformis	651.3	In the same family as 2 fish hosts

Rank <sup>b</sup>	Genus Mean Acute Value (µg/L)	Order	Species	SMAV adjusted to 100 hardness (µg/L)	Reason retained
9	80.38	Cypriniformes	Bonytail,  Gila elegans (LS)	80.38	In the same family as 5 fish hosts
8	76.02	Cypriniformes	Razorback sucker, Xyrauchen texanus (LS)	76.02	In the same order as 5 fish hosts
7	46.79	Cypriniformes	Colorado pikeminnow, Ptychocheilus lucius (LS)	46.79	In the same family as 5 fish hosts
-	-	Cypriniformes	Northern pikeminnow, Ptychocheilus oregonensis	N/A <sup>b</sup>	In the same family as 5 fish hosts
6	>15.72	Salmoniformes	Mountain whitefish, Prosopium williamsoni	>15.72	In the same family as 2 fish hosts
5	6.141	Salmoniformes	Cutthroat trout, Oncorhynchus clarkii	5.401	In the same family as 2 fish hosts
-	-	Salmoniformes	Coho salmon, Oncorhynchus kisutch (LS)	11.88	In the same family as 2 fish hosts
-	-	Salmoniformes	Rainbow trout, Oncorhynchus mykiss (LS)	3.727	In the same family as 2 fish hosts
-	-	Salmoniformes	Chinook salmon, Oncorhynchus tshawytscha (LS)	5.949	In the same family as 2 fish hosts
4	5.931	Perciformes	Striped bass,  Morone saxatilis	5.931	Direct host
3	5.642	Salmoniformes	Brown trout, Salmo trutta	5.642	Direct host
2	4.411	Scorpaeniformes	Mottled sculpin, Cottus bairdii	4.418	Direct host
-	-	Scorpaeniformes	Shorthead sculpin, Cottus confusus	4.404	In same genus as 2 fish hosts
1	4.190	Salmoniformes	Bull trout, Salvelinus confluentus	4.190	In the same family as 2 fish hosts
-	-	Salmoniformes	Brook trout, Salvelinus fontinalis	N/A <sup>b</sup>	In the same family as 2 fish hosts

<sup>&</sup>lt;sup>a</sup> Data were obtained from Table 7 of USEPA (2016). Fish species were retained if they were members of the same Order of a fish that may serve as a dwarf wedgemussel host according to USFWS (2019).

<sup>&</sup>lt;sup>b</sup> There is a 10x diff in SMAVs for the genus, only most sensitive SMAV is used in the calculation

Table 7. Dwarf wedgemussel host fish-specific acute HC5 calculations.<sup>a</sup>

N	Rank	GMAV	ln(GMAV)	ln(GMAV) <sup>2</sup>	P=R/(N+1)	sqrt(P)
22	4	5.931	1.78	3.17	0.174	0.417
	3	5.642	1.73	2.99	0.130	0.361
	2	4.411	1.48	2.20	0.087	0.295
	1	4.190	1.43	2.05	0.043	0.209
	Sum:		6.43	10.42	0.435	1.282

 $S^2 = 3.75$  L = 0.986 A = 1.419 FAV = 4.134**Acute HCs = 2.1** 

<sup>&</sup>lt;sup>a</sup> In accordance with USEPA (1985), calculations are based on the four most sensitive genera (see table 5).

Table 8. Ranked freshwater genus mean chronic values (GMCV) for species within the same order as dwarf wedgemussel fish host species.<sup>a</sup>

Rank <sup>b</sup>	Genus Mean Chronic (μg/L)	Order	Species	SMCV adjusted to 100 hardness (µg/L)	Reason retained
10	>38.66	Perciformes	Blue tilapia,  Oreochromis aureus	>38.66°	In the same order as 10 fish hosts
9	16.43	Perciformes	Bluegill, Lepomis <i>macrochirus</i>	16.43	Direct host
8	14.22	Perciformes	Smallmouth bass, <i>Micropterus dolomieu</i>	14.22°	In the same family as 3 fish hosts
7	14.16	Cypriniformes	Fathead minnow, Pimephales promelas	14.16	In the same family as 5 fish hosts
6	13.66	Cypriniformes	White sucker, Catostomus commersonii	13.66°	In the same order as 5 fish hosts
5	8.723	Cyprinodontiformes	Flagfish, Jordanella floridae	8.723	In the same order as 1 fish host
4	3.360	Salmoniformes	Atlantic salmon, Salmo salar	2.389	Direct host
-	-	Salmoniformes	Brown trout, Salmo trutta	4.725	Direct host
3	3.251	Salmoniformes	Rio Grande cutthroat trout,  Oncorhynchus clarkii virginalis	3.543	In the same family as 2 fish hosts
-	-	Salmoniformes	Coho salmon, Oncorhynchus kisutch	N/A <sup>b</sup>	In the same family as 2 fish hosts
-	-	Salmoniformes	Rainbow trout,  Oncorhynchus mykiss	2.192	In the same family as 2 fish hosts
-	-	Salmoniformes	Chinook salmon, Oncorhynchus tshawytscha	4.426	In the same family as 2 fish hosts
2	2.356	Salmoniformes	Brook trout, Salvelinus fontinalis	2.356	In the same family as 2 fish hosts
-	-	Salmoniformes	Lake trout, Salvelinus namaycush	N/A <sup>b</sup>	In the same family as 2 fish hosts
1	1.470	Scorpaeniformes	Mottled sculpin, Cottus bairdii	1.470	Direct host

<sup>&</sup>lt;sup>a</sup> Data were obtained from Table 9 of USEPA (2016). Fish species were retained if they were members of the same Order of a fish that may serve as a dwarf wedgemussel host according to USFWS (2019).

<sup>&</sup>lt;sup>b</sup> Not included in the GMCV calculation because normalized EC<sub>20</sub> data available for the genus.

<sup>&</sup>lt;sup>c</sup> Calculated from the MATC and not EC<sub>20</sub> but retained to avoid losing a SMCV.

Table 9. Dwarf wedgemussel host fish-specific chronic HC5 calculations.<sup>a</sup>

N	Rank	GMAV	ln(GMAV)	ln(GMAV) <sup>2</sup>	P=R/(N+1)	sqrt(P)
10	4	3.360	1.21	1.47	0.364	0.603
	3	3.251	1.18	1.39	0.273	0.522
	2	2.356	0.86	0.73	0.182	0.426
	1	1.470	0.39	0.15	0.091	0.302
	Sum:		3.63	3.74	0.909	1.853

 $S^2 = 8.75$ L = -0.462A = 0.199

Chronic  $HC_5 = 1.2$ 

<sup>&</sup>lt;sup>a</sup> In accordance with USEPA (1985), calculations are based on the four most sensitive genera (see table 5).